FINAL TECHNICAL REPORT

GRANT #: N00014-04-1-0756

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INSTITUTION: University of Tennessee

GRANT TITLE: Development of Hemostatic Agents

AWARD PERIOD: July 30, 2004 – August 1, 2005

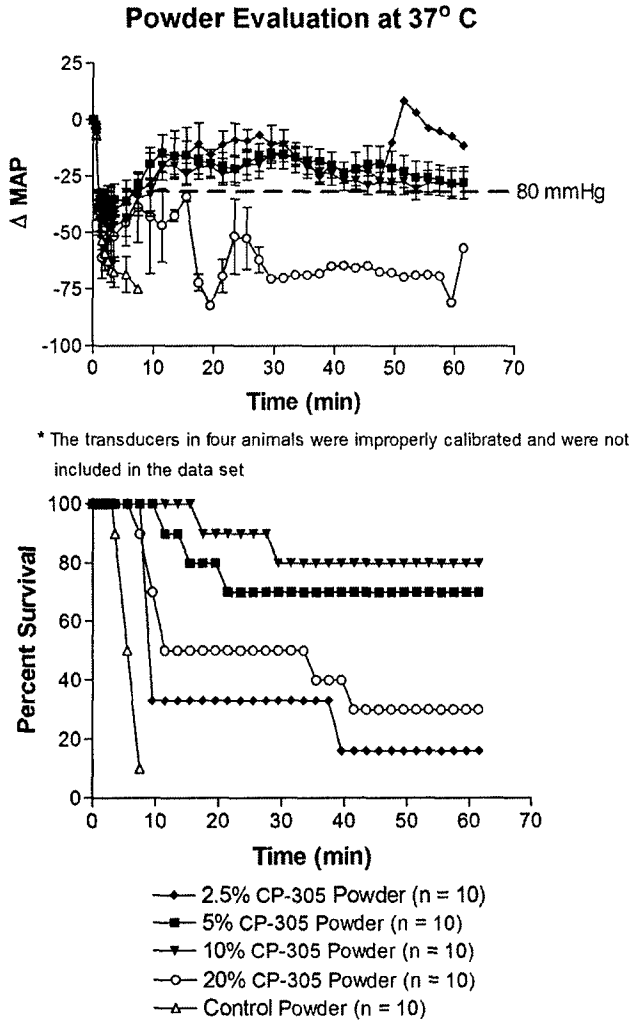
OBJECTIVE: To assess the efficacy of a series of novel hemostatic agents in normal and coagulopathic animals subjected to femoral artery hemorrhage. The hemostatic agents comprise different carrier matrices for the delivery of the lead compound CP-305. The objective set forth in this research was to identify candidate formulations that could ultimately prevent hypovolemia and exsanguination resulting from traumatic battlefield injuries.

APPROACH: The development of the hemostatic devices has centered on the rat model of lethal groin injury. Specifically, animals were subjected to femoral bundle transaction and hemorrhaged until a mean arterial pressure (MAP) of 40 mmHg was achieved. Hemostatic matrices containing graded doses of CP-305 in powder formulations comprised of protosan and excipients, and bilayer patch formulations containing protosan, excipients, a plasticizer, and an anti-oxidant were applied to the wound site. After a hold time of three minutes the integrity of the seal was assessed by reperfusion of the animal to a minimum MAP of 80 mmHg using 12 milliliters of lactated Ringers or less. Successful formulations were defined as those that do not leak for one hour at or above an MAP of 80 mmHg wherein 12 milliliters or less were used to achieve the target MAP. Furthermore, to assess the effects of coagulopathy the experiments were conducted at 30°C and compared to the results obtained at 37°C.

ACCOMPLISHMENTS: We successfully developed two carrier matrices for CP-305 consisting of a powder and a patch formulation. The first objective was to identify materials that possessed structural and wetting properties wherein the material did not rapidly dissolve in the wound nor wetted to slowly to facilitate the release of CP-305. A series of pilot studies using carbopol, protosan, carboxycellulose, and collagen were conducted from which protosan was selected as the structural element for the powder formulations. The investigations into lyophilized materials to form patches identified that a single material could not achieve structural integrity and a rapid enough dissolution rate for drug release. A bilayer system was adopted wherein protosan provides the mechanical integrity while a thin highly porous layer of collagen provides the release stage in the patch. The wetting properties and flexibility of the patches and the wetting of the powder were further optimized by the addition of excipients and plasticizers. The addition of an anti-oxidant to increase the thermal and air stability of CP-305 generated our prototype delivery matrices.

Phase two of the project was to determine the optimum loading of CP-305 that afforded hemostasis in the rodent model of groin injury. The powder matrix presented fewer technical challenges to formulate and as such was selected to for the initial dose ranging study. The hemostatic efficacy of CP-305 was evaluated using powder formulations containing 2.5, 5, 10, and 20 percent drug. In these studies animals maintained at 37°C were hemorrhaged to a MAP of 40 mmHg, treated with a dosage form, held for three minutes, and then resuscitated with lactated Ringers (Figure 1). The mean volume of shed blood to achieve an MAP of 40 mmHg was 5.2 milliliters and the mean resuscitation volume in a successful experiment was 7 milliliters and in unsuccessful experiments the total volume of lactated Ringers was 12 milliliters. As shown in Figure 1, the powder formulations containing 5 and 10 percent CP-305 were highly efficient in sealing the wound site as evidenced by the ability to perfuse the animal to a MAP of 80 mmHg or above with successful seal rates of 70 and 80 percent, respectively. The percent survival in the Kaplan-Meyer graph is a direct readout of the number of experiments that achieved hemostasis, *i.e.* in unsuccessful seals the animals continued to hemorrhage post hemostatic application resulting in a decline of the MAP below 20 mmHg. In contrast, the control powder fail to seal the wounds in all cases whereas 50 and 70 percent of the experiments failed using the 20 and 2.5 percent formulations, respectively, ten minutes post hemostatic treatment. These studies demonstrated that our hypothesis that CP-305 was a novel agents for hemostatic development as evidenced by the inability of the control powder to achieve hemostasis.

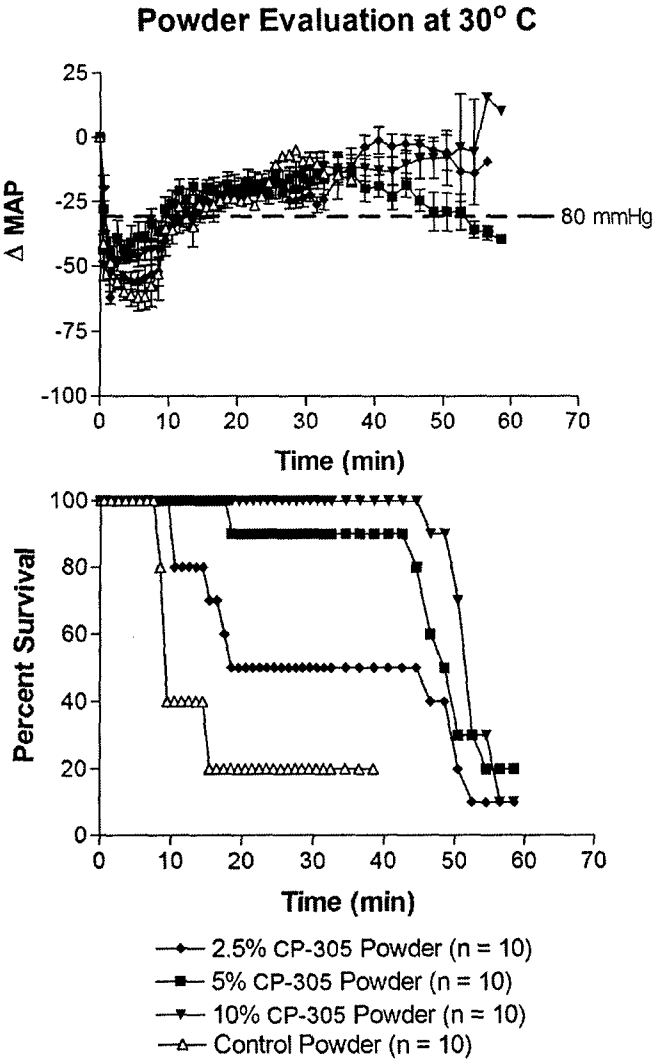
Figure 1



Under ideal conditions hemorrhage control is initiated immediately after injury; however, immediate treatment is seldom the situation encountered in battlefield conditions. The delayed response to hemorrhage control combined with the severity of battlefield injuries can lead to the development of hypovolemia and hypothermia. These factors can lead to coagulopathy thus compounding hemorrhage control efforts. To induce a coagulopathic state the powder hemostatic experiments were conducted on animals maintained at 30°C using 2.5, 5, and 10 percent powder formulations. The 2.5 percent loading was included to verify the dose range and the 20 percent was deleted due to the low efficacy and high drug loading. The results of the studies using the hemostatic powders in hypothermic animals are shown in Figure 2. In these studies the percent survival at 45 minutes is representative of the seal success. After 45 minutes all but 4 animals undergo hydrodynamic collapse possibly due to a reduced hematocrit and hypothermia. Post experiment necropsies did not reveal any gross lesions that would account for the circulatory

collapse. The results of these studies are consistent with the data collected at 37°C in that the 5 and 10 percent powders were successful in preventing further hemorrhage in 90 and 100 percent of the animals, respectively. The reason for the increase survival in the control and 2.5 percent CP-305 powders remain unclear; however, it is plausible that the reduced blood temperature slowed powder dissolution and/or wetting and/or increased blood viscosity aided wound sealing. The net results of the combined studies indicate that a dose range between 5 and 10 percent is optimal for achieving seal integrity for CP-305 in a powdered form. However, the use of a powdered agent in a battlefield environment is highly impractical thus requiring an alternate delivery device for CP-305.

Figure 2



The ideal device should maintain physical characteristic that are close to deployed hemostatic devices to prevent the need for retraining first responders. To this end a patch or granular formulation is highly desirable based on currently deployed hemostatic agents. In this phase of development elected to develop a CP-305 loaded patch, a hemostatic form commonly utilized by front line responders. Pilot studies quickly identified protosan, purified chitosan, as the best matrix based on material cost, drug release, and thermal stability; however, the material alone possessed several unwanted characteristics including: 1) the lyophilized sponges of protosan and CP-305 were brittle; 2) formulations that readily released the drug gelled on contact with the wound and were expelled and; 3) matrices that possessed slow dissolution also retarded drug release. The solution to these problems was overcome by creating a hybrid delivery system for CP-305. The addition of excipients and a plasticizer eliminated the brittle characteristics of the patch and enhanced wetting; however, a patch with sufficient mechanical stability resulted in a patch with slow CP-305 release properties. This drawback was overcome by the addition of a thin highly porous collegen-CP-305 layer over the core of the patch. The rationale for the design was that collagen would be readily dissolved by the blood releasing the drug and may also enhance blood clotting. Attempts to use protosan in a similar structure resulted in gelling and entrapment of CP-305. Utilizing this design we prepared patch formulations containing 5, 10, and 20 percent CP-305 and evaluated their efficacy in the rat model of groin injury at 37°C (Figure 3). In these experiments the 10 percent patch sealed 80 percent of the wounds whereas the 5 and 20 percent patches yielded a 70 percent success rate. The control patches containing protosan and collagen had a rapid and high failure rate with none of the animals surviving the length of the experiment. The successful seals were also

Figure 3

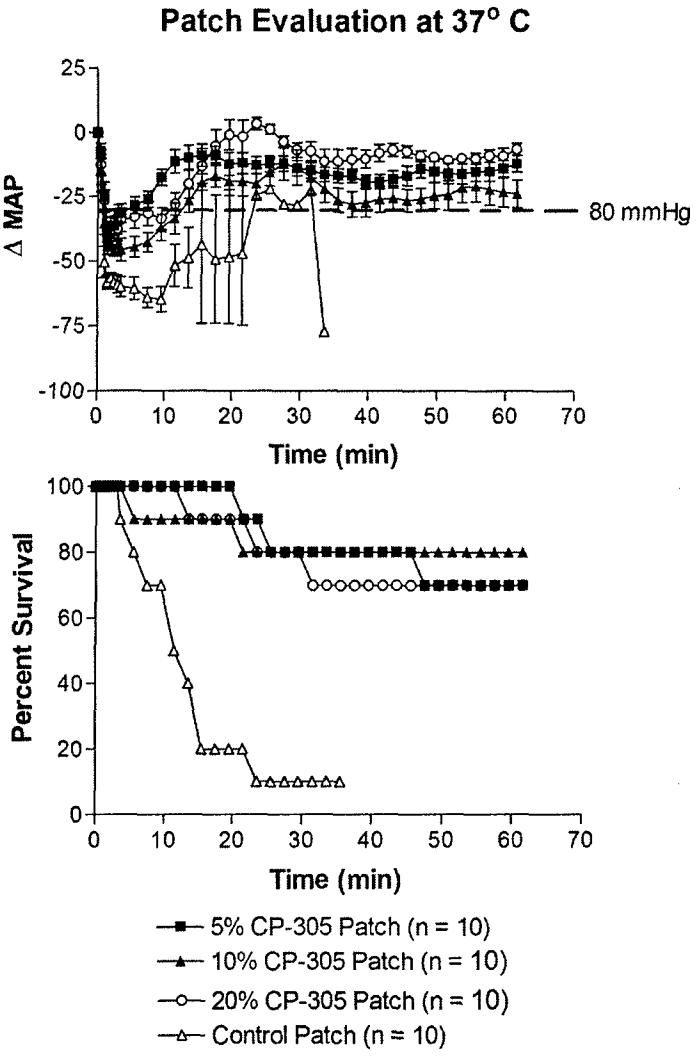
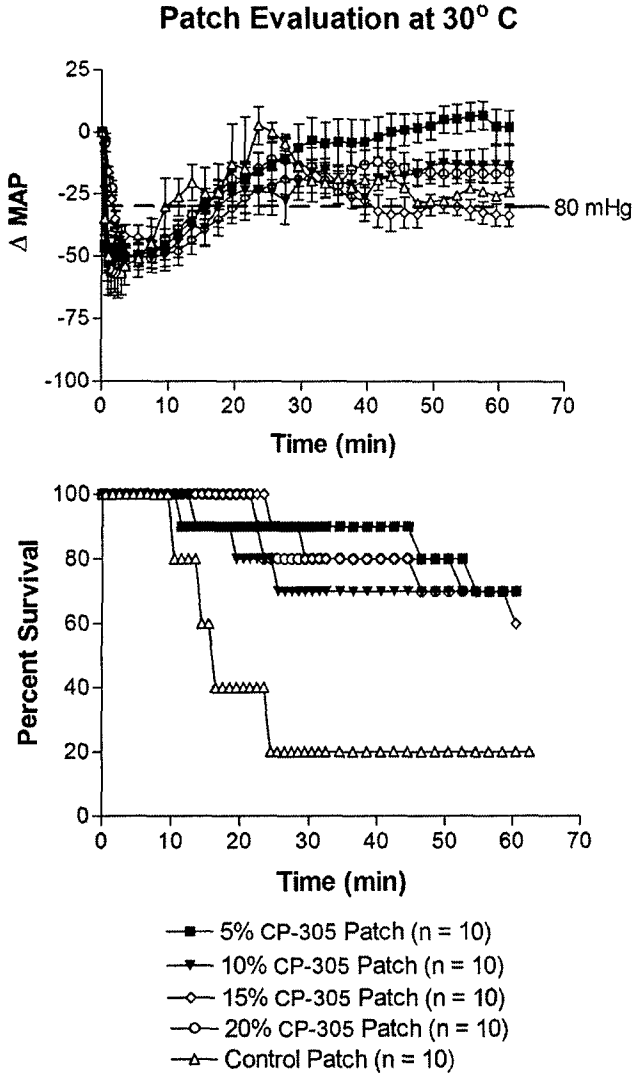


Figure 4



very stable as evidenced by the re-perfusion pressures ranging from 83 to 97 mmHg. An additional finding from these studies is that the addition of a tin film of collagen is not contributing to the hemostatic properties, again demonstrating the hemostatic efficacy of the lead drug CP-305.

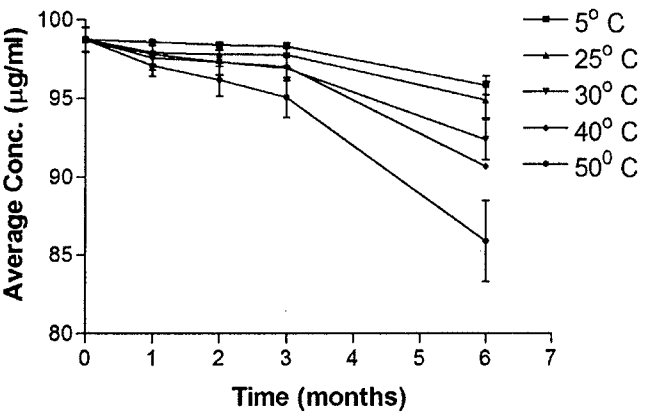
The experiments were repeated in animals maintained at 30°C for the reasons previously stated. To better analyze the dose window patches employing 5, 10, 15, and 20 percent CP-305 were studied (Figure 4). The 5, 10, and 15 percent patches sealed the wound in seven of the ten animals whereas the 20 percent patches provided hemostasis in 6 of the animals. Two of the control animals survived the length of the experiment possibly due to the aforementioned factors discussed for the experiments on the powder formulations.

The results of the studies on the patch and powder formulations in animals maintained at 37°C and 30°C strongly support the efficacy of the lead compound CP-305 as a hemostatic agent. The studies have identified that 5, 10, and 15 percent formulations are optimum for wound sealing; however, the patches require further optimization to increase the efficacy of the devices. The success of the powder formulation has prompted us to investigate formulating CP-305 into a granular matrix. A

granular matrix may in fact increase the efficacy because of the ability of granules to flow through the wound bed.

A critical element to the successful deployment of a CP-305 based hemostatic agent is the thermal stability of the active ingredient. Deployed hemostatic agents will be subjected to a wide range of temperature. It is therefore essential to assess the thermal stability of CP-305 at temperatures ranging from 5 to 50°C. Stability studies were conducted on the 10 percent powder formulation over the course of six months (Figure 5). All analysis were carried out in triplicate and validated against a standard curve. The studies show that the CP-305 powder is relatively stable at temperatures for 30°C or below; however, after 6 months at 45 and 50°C there was a 10 and 15% loss of active drug in the samples. These results prompted the inclusion of an anti-oxidant into the patch formulations.

Figure 5



CONCLUSIONS: Our research has conclusively demonstrated that CP-305 possesses potent hemostatic activity. In powder and patch formulations, concentrations of CP-305 ranging from 5 to 15 percent by weight exhibit from 70 to 100 percent seal rates under re-perfusion MAPs ranging from 80-100 mmHg. These studies have laid the essential ground work for translating the novel hemostatic agents into the swine model of lethal groin injury from which clinically relevant devices will be developed.

SIGNIFICANCE: Our studies have identified a highly efficacious hemostatic agent that will be developed as a life saving device for treating life threatening battlefield hemorrhage.

PATENT INFORMATION: A patent application is pending on the use of CP-305 and related compounds for hemorrhage control. U.S. Patent Application Serial No. 10/411,479 "Method and Kit for Controlling Bleeding" Filed: April 8, 2003. B.M. Moore II, D. D. Miller.

AWARD INFORMATION: MBJ Health Care Hero Award for Innovation (2005)

REFEREED PUBLICATIONS (for total award period): None

BOOK CHAPTERS, SUBMISSIONS, ABSTRACTS AND OTHER PUBLICATIONS (for total award period)

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